Lack of regulation of 11 beta-hydroxysteroid dehydrogenase type 1 during short-term manipulation of GH in patients with hypopituitarism

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CLINICAL STUDY

Lack of regulation of 11β-hydroxysteroid dehydrogenase type 1 during short-term manipulation of GH in patients with hypopituitarism

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Abstract

Objective: Evidence from long-term clinical studies measuring urinary steroid ratios, and from *in vitro* studies, suggests that GH administered for longer than 2 months down-regulates 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), thereby reducing cortisol regeneration in liver and adipose tissue. We aimed to measure acute effects of GH on 11β-HSD1 in liver and adipose tissue *in vivo*, including using a stable isotope tracer.

Design: Observational studies of GH withdrawal and reintroduction in patients with hypopituitarism.

Methods: Twelve men with benign pituitary disease causing GH and ACTH deficiency on stable replacement therapy for >6 months were studied after GH withdrawal for 3 weeks, and after either placebo or GH injections were reintroduced for another 3 weeks. We measured cortisol kinetics during 9,11,12,12-$^{2}H_4$-cortisol (d4-cortisol) infusion, urinary cortisol/cortisone metabolite ratios, liver 11β-HSD1 by appearance of plasma cortisol after oral cortisone, and 11β-HSD1 mRNA levels in subcutaneous adipose biopsies.

Results: GH withdrawal and reintroduction had no effect on 9,12,12-$[^{2}H]_3$-cortisol (d3-cortisol) appearance, urinary cortisol/cortisone metabolite ratios, initial appearance of cortisol after oral cortisone, or adipose 11β-HSD1 mRNA. GH withdrawal increased plasma cortisol 30–180 min after oral cortisone, increased d4-cortisol clearance, and decreased relative excretion of 5α-reduced cortisol metabolites.

Conclusions: In this setting, GH did not regulate 11β-HSD1 rapidly *in vivo* in humans. Altered cortisol metabolism with longer term changes in GH may reflect indirect effects on 11β-HSD1. These data do not suggest that glucocorticoid replacement doses need to be increased immediately after introducing GH therapy to compensate for reduced 11β-HSD1 activity, although dose adjustment may be required in the longer term.

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Introduction

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) regenerates cortisol from inactive cortisone in liver and adipose tissue. It has been proposed that GH, via insulin-like growth factor-1 (IGF1), down-regulates 11β-HSD1 (1, 2, reviewed in (3)); that increased 11β-HSD1 in GH-deficient adults may contribute to their central obesity; and that introducing GH treatment in hypopituitary patients may decrease 11β-HSD1 and precipitate adrenocortical insufficiency (4) or necessitate an alteration in glucocorticoid replacement (5, 6).

A key question is whether changes in 11β-HSD1 occur acutely after introducing GH therapy. *In vitro*, IGF1 down-regulates 11β-HSD1 expression rapidly in adipocytes (2, 7) but not in hepatocytes (8). However, *in vivo* studies in GH-deficient patients have measured 11β-HSD1 after a minimum of 2 months of therapy (1, 5, 6, 9–12), when indirect effects may affect 11β-HSD1. In acromegaly (2, 13), changes in urinary cortisol metabolite ratios following withdrawal of octreotide occur after 4–8 weeks (2). In obese men, GH effects on 11β-HSD1 were observed after 6 weeks but resolved after 4–9 months (14, 15).

Previous studies of GH effects on 11β-HSD1 *in vivo* have relied almost exclusively on measuring urinary ratios of cortisol/cortisone metabolites; these are influenced by enzymes other than 11β-HSD1, notably 5α- and 5β-reductase, and have not been validated in patients receiving glucocorticoid replacement therapy. Although in one study GH administration reduced plasma cortisol after oral cortisone administration (6),

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plasma cortisol was also lower after oral hydrocortisone, suggesting mechanisms in addition to inhibition of 11β-HSD1. However, in another study, GH reduced 11β-HSD1 mRNA in adipose tissue (11).

To test the hypothesis that GH/IGF1 regulates 11β-HSD1 directly and acutely *in vivo* in human liver and adipose tissue, we studied patients with hypopituitarism during long-term GH therapy, after GH withdrawal for 3 and 6 weeks and after GH reintroduction for 3 weeks. To quantify the rate of cortisol regeneration by 11β-HSD1 we used a steady-state tracer infusion with 9,11,12,12-$^2H_4$-cortisol (d4-cortisol) (16). During d4-cortisol infusion, there is removal of the 11α-^{2}H by 11β-HSD type 2 to form 9,12,12-[^{2}H]$_3$-cortisone (d3-cortisone) which is then regenerated to 9,12,12-[^{2}H]$_3$-cortisol (d3-cortisol) by 11β-HSD1; d4-cortisol is not regenerated by 11β-HSD1 since it is highly unlikely that a deuterium (^{2}H) will be reintroduced in the 11α position. The dilution of d4-cortisol by d3-cortisol therefore indicates 11β-HSD1 reductase activity. We also measured liver 11β-HSD1 activity (by first pass conversion of orally administered cortisone to cortisol) and adipose 11β-HSD1 mRNA (in subcutaneous adipose tissue biopsies).

Subjects and methods

Patients

Inclusion criteria: male; hypopituitarism due to benign disease; age 20–75 years; documented GH and ACTH deficiency; stable hormone replacement therapy, including GH and glucocorticoid for >6 months, achieving normal range serum IGF1. Exclusion criteria: acromegaly; anti-inflammatory glucocorticoid therapy by any route within 3 months; significant co-morbidity. Informed consent and local ethical committee approval were obtained.

Protocol

Participants were randomly allocated to either of the two groups. Six men were studied taking their usual therapy ('baseline'), 3 weeks after withdrawal of GH therapy, and 3 weeks after reintroduction of GH therapy. One man withdrew after completing baseline and some measurements after 3 weeks of GH withdrawal. Another six men were studied at baseline, 3 weeks after withdrawal of GH therapy, and 6 weeks after withdrawal of GH therapy; placebo was administered during weeks 3–6.

Measurements were made during three visits within the final 5 days of each phase. In one visit, a 24-h urine sample was collected; a blood sample was obtained at 0800 h after overnight fast and usual medication at 0700 h; anthropometric measurements were recorded; dual-energy X-ray absorptiometry was measured for body composition (LUNAR DPX-L scanner, Scanexport Medical, Helsingborg, Sweden); and a ~300 mg gluteal subcutaneous adipose biopsy was obtained under local anaesthesia. On a second visit, after overnight fast and omission of morning medication, a single dose of 25 mg cortisone acetate was administered and plasma cortisol measured at intervals for 3 h. On a third visit, after overnight fast and omission of morning medication, d4-cortisol (Cambridge Isotopes, Andover, MA, USA) was infused at 20% molar percent excess in cortisol at 1.74 mg/h and blood sampled at intervals for 5 h.

Laboratory assays

Cortisol and its metabolites (5β-tetrahydrocortisol (THF), 5α-THF, 5β-tetrahydrocortisone, cortols, cortolones, and cortisone) were measured in 24-h urine samples by gas chromatography/mass spectrometry as previously described (17, 18).

Plasma d4-cortisol, d3-cortisone and d3-cortisol were measured by gas chromatography/mass spectrometry as previously described (18) and tracer kinetics calculated using the mean of up to six measurements in steady state between 225 and 300 min of d4-cortisol infusion. Clearance of d4-cortisol was calculated as (infusion rate)/(concentration). Rate of appearance of d3-cortisol was calculated as (d4-cortisol infusion rate)/(d4-cortisol:d3-cortisol ratio).

RNA was extracted from 100 mg of tissue, oligo dT-primed cDNA synthesised, and transcripts quantified in triplicate using Real-Time PCR with a LightCycler 480 and Mastermix (Roche) and primer-probe sets from Applied Biosystems (Cheshire, UK), as previously described (19). Results are expressed as a ratio of transcript level to the mean of cyclophylin A and 18S.

Plasma cortisol was measured by RIA (ImmunChem, MP Biomedical, High Wycombe, UK), immunoluminetric methods were used to measure serum IGF1 (Nichols Advantage Specialty System, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) and insulin (ADVIA Centaur Insulin Ready Pack, Bayer Corporation). Enzymatic methods were used to measure plasma glucose (GLU, Roche/Hitachi Roche Diagnostics GmbH), free fatty acids (FFA; NEFA C, WAKO Chemicals GmbH, Neuss, Germany) and triglycerides (TG, Roche/Hitachi Roche Diagnostics GmbH).

Statistical analysis

A power calculation showed that a study of $n=6$ had 85% power ($n=12$ has >98% power) to detect an 18% change in d3-cortisol generation in a paired two-tailed test, based on previously published variance of measurement (16, 18) and magnitude of effect of GH on cortisol levels after oral cortisone administration (6). Data are mean ± S.E.M. Groups were compared at baseline by unpaired Student's *t*-tests. Effects of GH withdrawal and reintroduction were assessed by one-way repeated measures ANOVA within each group of six patients followed by *post hoc* paired Student's *t*-tests, if appropriate. Furthermore, the effect of the 3-week

withdrawal of GH was tested in a combined analysis of 'baseline' and the '3-week withdrawal' data in all 12 patients using paired Student's *t*-tests. Multiple cortisol measurements after oral cortisone administration were analysed by factorial repeated measures ANOVA, followed by *post hoc* paired *t*-tests at individual time points, if appropriate; curves were also fitted using Kinetica software (Thermo Scientific, Waltham, MA, USA) to derive an initial appearance rate constant (K_a).

Results

Patients had hypopituitarism after removal of benign pituitary adenoma ($n=11$) or craniopharyngioma ($n=1$). All patients were receiving substitution therapy with glucocorticoids (10 hydrocortisone, 2 cortisone acetate), thyroxine and testosterone. Five patients were receiving desmopressin. At baseline, the group randomly selected for prolonged GH withdrawal had higher

Table 1 Effects of GH withdrawal and re-introduction.

	Study group 1			Study group 2		
	Baseline	3 week withdrawal	3 week reintroduction[a]	Baseline	3 week withdrawal	6 week withdrawal
n	6	6	5	6	6	6
Age (years)	64.0±2.1			64.5±4.8		
Height (cm)	177.4±3.2			178.6±2.4		
GH dose (mg/d)	0.4±0.2	0	0.4±0.2	0.3±0.2	0	0
IGF1 (ng/ml)	153.2±16.0	57.5±8.7§	152.4±19.7¶	180.5±28.6	78.2±15.7‡	72.8±12.2§
Weight (kg)	89.0±2.9	88.6±3.20	88.5±3.7	102.8±9.2	102.4±9.2	102.5±8.9
BMI (kg/m^2)	28.5±1.6	28.5±1.6	27.2±1.5	32.2±2.6	32.3±2.6	32.0±2.6
Waist (cm)	100.6±5.2	102.3±4.7	98.0±2.8	108.2±6.1	107.7±5.5	105.2±6.2
Hip (cm)	103.8±1.5	104.4±1.8	102.8±0.6	110.2±4.1	109.8±3.9	108.4±4.3
Waist-to-hip ratio	0.97±0.04	0.98±0.03	0.95±0.03	0.98±0.02	0.98±0.02	0.97±0.02
DEXA % fat	25.9±3.6	26.1±4.1	21.9±2.1	28.9±2.7	30.0±3.2	30.3±2.6‖
DEXA lean mass (kg)	61.8±2.9	61.5±3.2	65.2±3.2	68.3±4.3	66.0±3.1	66.5±3.8
Systolic BP (mmHg)	140±6	145±7	137±6	145±10	142±8	138±10
Diastolic BP (mmHg)	81±2	83±2	80±3	85±5	83±5	81±5‡
Fasting plasma						
Glucose (mM)	4.75±0.25	4.60±0.23‡	4.42±0.24	5.02±0.30	4.88±0.53	4.45±0.21‡
Insulin (mU/l)	6.38±2.55	3.52±0.48	3.66±0.49	11.52±4.01	15.92±11.29	8.20±2.58
FFA (mM)	0.51±0.08	0.40±0.09	0.37±0.06	0.59±0.05	0.40±0.03‡	0.35±0.03§
Triglycerides (mM)	0.84±0.07	0.76±0.05	0.99±0.10	2.27±0.52*	1.90±0.52	1.77±0.49§
24 h urine[b]						
Total cortisol metabolites (mg/d)	9.84±3.17	11.10±2.9	14.2±2.3	10.3±1.9	9.5±0.8	9.5±1.8
(5αTHF+5βTHF)/THE	2.33±0.79	8.07±2.56	8.08±3.42	5.55±2.59	6.34±2.63	5.20±3.13
Cortisol/cortisone	9.74±4.65	5.67±1.42	4.98±1.02	8.93±5.77	5.24±2.20	8.22±3.36
5αTHF/5βTHF	1.06±0.11	1.02±0.12	1.15±0.09	1.62±0.29	1.47±0.18	1.20±0.33
5αTHF/cortisol	8.03±1.39	9.41±1.72	9.57±0.69	11.73±1.67	11.48±2.19	8.87±2.31‡
5βTHF/cortisol	7.82±1.39	9.09±1.16	8.24±0.74	7.73±1.13	8.56±1.94	8.35±1.43
d4-Cortisol tracer kinetics						
d4-Cortisol clearance (l/min)	0.73±0.29	1.93±1.37	0.79±0.22	0.65±0.14	0.66±0.16	0.86±0.21‡
Ra d3-cortisol (nmol/min)	15.3±1.0	16.1±2.5	13.4±0.9	6.5±0.9†	7.4±1.4	6.8±1.1
Plasma cortisol after oral cortisone						
Appearance rate constant (K_a, per min)	0.074±0.019	0.031±0.008	0.046±0.014	0.061±0.031	0.041±0.012	0.073±0.024
Adipose mRNA						
11β-HSD1	0.55±0.09	0.65±0.17	0.65±0.20	0.69±0.14	0.64±0.14	0.79±0.25
Hexose-6-phosphate dehydrogenase	0.75±0.07	0.73±0.08	0.59±0.03	0.71±0.09	0.78±0.07	0.69±0.09
GRα	0.80±0.08	0.77±0.09	0.74±0.05	0.83±0.09	0.73±0.07	0.86±0.09
5α-Reductase 1	0.94±0.10	1.12±0.15	1.23±0.34	1.00±0.07	0.92±0.13	1.18±0.17

*$P<0.05$, †$P<0.01$ for differences between study groups at baseline; ‡$P<0.05$, §$P<0.01$, ‖$P<0.001$ for effects of GH withdrawal (i.e. differences from baseline) within each study group; ¶$P<0.01$ for effects of GH reintroduction (i.e. differences from 3 week withdrawal) in study group 1. BMI, body mass index; BP, blood pressure; FFA, free fatty acids; Ra, rate of appearance; GR, glucocorticoid receptor.

[a]Note that one subject withdrew from the GH reintroduction group, so that mean data may appear to differ from values after 3 weeks withdrawal, but only statistically significant differences are highlighted.

[b]Total cortisol excretion was calculated from the sum of 5β-THF, 5α-THF and THE. The balance between 11β-HSD activities in all tissues was assessed as the ratio of (5β-THF +5α-THF)/THE. Renal 11β-HSD type 2 activity was assessed as urinary cortisol/cortisone ratio. The balance of 5α- and 5β-reductases was assessed by the ratio 5β-THF/5α-THF. 5α- and 5β-reduction of cortisol was also assessed by 5α-THF/cortisol and 5β-THF/cortisol ratios.

fasting plasma triglycerides and tended to have higher body mass index, % body fat and fasting insulin levels than the group selected for GH withdrawal followed by reintroduction (Table 1).

Withdrawal of GH resulted in a substantial fall in IGF1 after 3 weeks which was sustained after 6 weeks. Reintroduction of GH restored IGF1 to baseline values within 3 weeks (Table 1). Withdrawal of GH after 3 weeks also reduced fasting plasma FFA (in all 12 participants, from 0.55 ± 0.05 to 0.40 ± 0.04 mM, $P=0.02$) and triglycerides (in all 12, from 1.55 ± 0.33 to 1.32 ± 0.30 mM, $P=0.04$), which were lower after 6 weeks of GH withdrawal (Table 1). Reintroduction of GH prevented this decline in triglycerides. After 6 weeks of GH withdrawal the percentage of body fat increased and diastolic blood pressure decreased, but there were no other measured changes in glucose/insulin homeostasis or body composition.

With exogenous glucocorticoid therapy, urinary cortisol metabolite excretion was highly variable (Table 1). There were no differences between groups in indices of overall 11β-HSD activity or renal 11β-HSD2 activity. However, the ratio of 5α-THF/cortisol, which reflects 5α-reductase activity in the liver, was decreased after 6 weeks GH withdrawal; the reintroduction of GH prevented this decline.

Kinetics of d4- and d3-cortisol is shown in Table 1 and Fig. 1A. By chance, d3-cortisol generation rate was higher at baseline in the group randomised to GH reintroduction. The d4-cortisol clearance increased after 6 weeks of GH withdrawal. The d3-cortisol regeneration rate was not affected by GH withdrawal or reintroduction in either group or when all 12 participants were included.

Cortisol levels following oral cortisone were higher after 3 weeks of GH withdrawal among all participants (Fig. 1B), and tended to rise further after 6 weeks GH withdrawal and revert to baseline values on reintroduction of GH. However, these changes occurred between 30 and 180 min and did not reflect the initial rate of appearance of cortisol <25 min after cortisone administration.

Adipose mRNA transcripts for 11β-HSD1 were unaffected by GH withdrawal or reintroduction (Table 1). Similarly, there were no changes in transcript levels of other genes thought to influence glucocorticoid signalling in adipose tissue: hexose-6-phosphate dehydrogenase, GRα and 5α-reductase type 1.

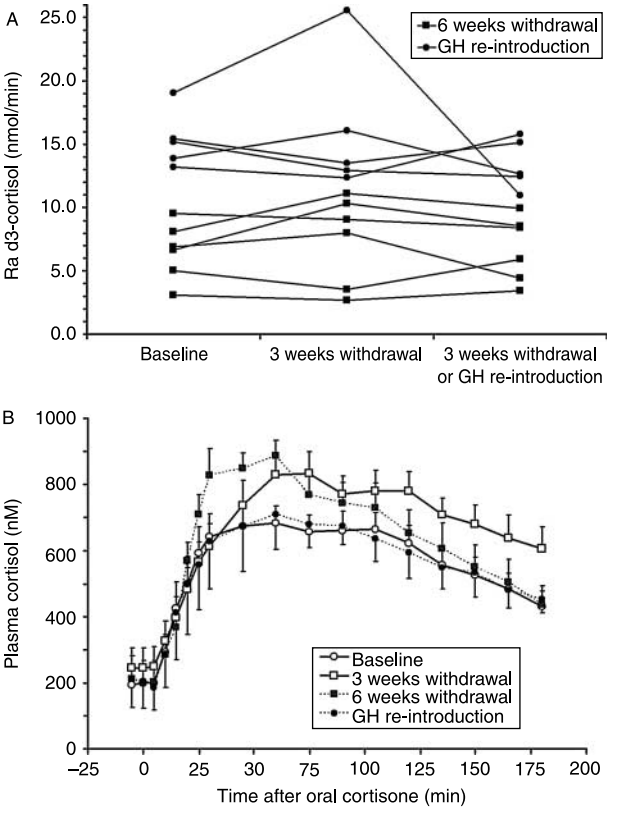

Figure 1 Effects of GH withdrawal and re-introduction on 11β-HSD1 conversion of cortisone to cortisol. (A) Rate of appearance (Ra) of d3-cortisol during steady-state d4-cortisol infusion. Results are shown for individual patients allocated to 6 weeks GH withdrawal (squares) or GH withdrawal and reintroduction (circles). Summary statistics are shown in Table 1. Results varied widely among patients with hypopituitarism but by chance, Ra of d3-cortisol was higher in the group allocated with GH reintroduction. However, there were no significant changes in Ra of d3-cortisol on GH withdrawal for 3 or 6 weeks, or on GH reintroduction for 3 weeks. (B) First pass conversion of cortisone to cortisol in liver. Results show mean ±S.E.M. plasma cortisol concentrations after oral administration of cortisone acetate 25 mg at time=0. Results were compared by repeated measures ANOVA and *post hoc* paired Student's *t*-tests, if appropriate. In all participants ($n=12$), there was a difference between baseline (open circle, solid line) and 3 weeks of GH withdrawal (open squares, solid lines; $P<0.03$; *post hoc* tests significant at time =150 and 180). There was no additional significant change in plasma cortisol after 6 weeks GH withdrawal ($n=6$; filled squares, dotted lines). However, cortisol values were reduced by reintroduction of GH treatment ($n=6$; filled circles, dotted lines; $P<0.02$ by ANOVA, no single time-points significant in *post hoc* tests). Curves from each individual were fitted with Kinetica software: the appearance rate constants (K_a) are shown in Table 1 and did not differ between groups.

Discussion

This is the first study to dissect acute effects of GH on 11β-HSD1 using specific *in vivo* assays in humans. It shows that in men with hypopituitarism, withdrawal of GH for up to 6 weeks, or reintroduction of GH for 3 weeks, has no effect on 11β-HSD1 in the whole body (d3-cortisol appearance rate and urinary cortisol/cortisone metabolite ratios), in liver (initial rate of cortisol appearance following oral cortisone) or in subcutaneous adipose tissue (transcript levels). These findings do not support the inference from previous long-term studies that GH acts through IGF1 directly to suppress 11β-HSD1 (3).

GH withdrawal increased plasma cortisol concentrations for 30–180 min after oral cortisone, but the lack of change in total urinary cortisol metabolite excretion suggests that the consequences for cortisol bioavailability are minor. It appears that the elevated plasma cortisol depends on factors other than 11β-HSD1 activity, since the initial rate of cortisol appearance was unaltered, and there was no associated change in d3-cortisol generation. 5α-Reductase activity (assessed by urinary 5α-THF/cortisol ratio) decreased, and d4-cortisol clearance paradoxically increased, on prolonged GH withdrawal, suggesting that GH affects other cortisol-metabolising enzymes. Alternatively, GH may influence gastric emptying (20).

GH withdrawal for 6 weeks was associated with a fall in plasma FFA and triglycerides and modest increase in body fat content without measurable changes in glucose/insulin homeostasis. This occurred in the absence of changes in 11β-HSD1, making it less likely that GH effects on 11β-HSD1 are a primary mediator of abnormal body composition in GH deficiency. Changes in 11β-HSD1 mRNA in adipose tissue (11) with prolonged GH therapy may reflect indirect effects; although these might perhaps be mediated by alterations in body composition and insulin signalling, as we have described in subjects receiving PPARγ agonists (19), previous studies have not associated changes in urinary cortisol/cortisone metabolite ratios with changes in insulin sensitivity or body fat content (9, 10).

The strength of these studies lies in the detailed assessment of cortisol metabolism and the paired analyses of 12 patients, which is a comparable sample size to that used in most previous studies in this area, and which we calculated gives ample statistical power to detect differences of the magnitude observed in previous studies. Weaknesses include: the fact that this group of patients had been exposed to chronic GH therapy in advance of the study, and GH was only deficient for 3 weeks, so that effects of GH (re)introduction may have been underestimated; the sample size was small for the subgroups at 6 weeks ($n=6$ and 5 per group); and the chance differences at baseline between these groups, e.g. in d3-cortisol generation and plasma triglycerides. As a result, direct comparisons at week 6 between subjects with and without GH reintroduction were avoided. The d4-cortisol tracer has been used extensively in healthy volunteers, but not in patients with glucocorticoid deficiency; we elected to limit our analysis to results which are entirely derived from the exogenous deuterated tracer infusion, and have not described the more variable results for endogenous cortisol production and metabolism.

Extrapolation from small exploratory studies must always be cautious, but an important clinical implication of these findings is that these data do not support the concept that the dose of glucocorticoid replacement therapy needs to be adjusted upwards in the short-term following introduction of GH to compensate for a fall in 11β-HSD1 activity. Although glucocorticoid dosing should be reassessed after medium to long-term GH therapy, further studies are required to clarify the need for dose adjustment in the short term.

Declaration of interest

B R Walker is an inventor on patents owned by the University of Edinburgh relating to 11β-HSD1 inhibitors. The other authors have no conflicts of interest to declare.

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